NURDs are required for diversity

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The EMBO Journal (2012) 31, 3036–3037. doi:10.1038/emboj.2012.137; Published online 11 May 2012

Embryonic stem cells (ESCs) can maintain their undifferentiated state indefinitely in culture (self-renewal), but must also retain the ability to differentiate into the various cell types found in the body (pluripotency). The co-existence of these two properties requires a balance between stable maintenance of ESC identity and the flexibility to reprogram cellular identity upon receiving a differentiation signal. The mechanisms underlying this developmental flexibility are largely unknown. Recently, Reynolds *et al* (2012) demonstrated that the Nucleosome Remodeling and Deacetylation (NURD) complex helps to maintain the developmental potency of ESCs by enforcing variability in the expression of transcription factors (TFs) that favour self-renewal, resulting in a low-expressing subpopulation of cells that are primed for differentiation. Thus, maintenance of ESC TFs at adequate, but not excessive, levels is important for maintaining ESC pluripotency.

In addition to their well-known ability to develop into the many cell types that are found in adults, ESCs are notable for other unusual features, such as their requirement to grow in colonies rather than as single cells. ESCs growing in colonies exhibit cell-to-cell phenotypic variation and, even under pluripotency-promoting conditions, ESC colonies include cells undergoing early differentiation processes. Interestingly, sub-populations of ESCs that express either high or low levels of several key effectors of self-renewal have been observed. Although cells expressing low levels of these factors are generally more prone to differentiation, these cells can still give rise to high-expressing cells within an ESC colony, indicating that, under pluripotency conditions, these states are reversible. A key step in understanding the biology of ESCs will be to understand how phenotypic variation is maintained and regulated in ESC colonies. In many organisms, variation in gene expression is associated with genes subject to regulation by chromatin-regulating machinery.

The NURD complex was originally identified as a histone deacetylase complex that also harbours ATP-dependent nucleosome remodelling activity (Tong *et al*, 1998; Xue *et al*, 1998). NURD was subsequently shown to be required for gene regulation and differentiation in multiple cell types, including ESCs, and to play a role in cancer development (Lai and Wade, 2011). NURD is necessary for normal ESC pluripotency, as homozygous deletion of the gene encoding

its Mbd3 subunit in mouse ESCs results in defects in differentiation (Kaji *et al*, 2006). Furthermore, loss of function of Mbd3 predisposes ESCs to differentiate into trophoblast, an extraembryonic cell type not normally produced by ESCs, highlighting the pluripotency defect conferred by Mbd3 loss (Kaji *et al*, 2006; Zhu *et al*, 2009).

Figure 1 NURD promotes transcriptional heterogeneity and facilitates ESC differentiation. (A) NURD promotes binding of PRC2 complex and facilitates repression of PRC2 targets. At some NURD and PRC2 target genes, NURD-dependent repression is opposed by transcriptional activators esBAF and Stat3, resulting in a bimodal expression pattern (see below). NURD is recruited to these targets in part by 5hmC. (B) NURD helps establish heterogeneity of pluripotency TF expression, which may in turn facilitate ESC differentiation. NURD promotes formation of a sub-population of cells that exhibit reduced expression of some pluripotency TFs, thus priming these cells for differentiation. This function is opposed by the actions of Stat3 (and esBAF) in media promoting self-renewal (+LIF), but is unopposed in conditions promoting differentiation (–LIF), in which Stat3 is inactive. Importantly, the inability of *Mbd3* knockout ESCs to differentiate normally is partially rescued by knockdown of factors such as *Klf4*, showing that the inability to repress these genes is at least partially responsible for the differentiation defects of ESCs lacking NURD.

However, until recently, the role of NURD in maintenance of the ESC gene regulatory network was not understood.

In a recent report, Hendrich and colleagues showed that deacetylation of H3K27 by the NURD complex is required for the binding and activity of the H3K27 methylase polycomb repressive complex 2 (PRC2) in mouse ESCs (Reynolds *et al*, 2011). NURD appears to be recruited to its targets in part via interaction of its Mbd3 subunit with the modified DNA base 5-hydroxymethylcytosine (5hmC), an oxidation product of the well-studied epigenetic mark, 5-methylcytosine (5mC) (Yildirim *et al*, 2011). Thus, one major function of the NURD complex appears to be repression, in combination with polycomb complexes, of 5hmC-marked genes (Figure 1A). Furthermore, the NURD complex also plays an additional role in gene regulation by deactivating enhancers that promote pluripotency gene expression during ESC differentiation (Whyte *et al*, 2012). While these studies address fundamental questions about the roles of NURD in ESC gene regulation, a key question remains: why do NURD mutants fail to differentiate properly?

A second study from Hendrich and colleagues recently published in *Cell Stem Cell* has begun to answer this question. Reynolds *et al* find that the NURD complex functions in ESCs to dampen expression of genes encoding key pluripotency TFs Klf4, Klf5, Zfp42 and Tbx3 by opposing the actions of transcriptional activators of these genes (Reynolds *et al*, 2012). Wild-type ESCs exhibit bimodal expression of these factors, and this phenotypic variation requires Mbd3, as *Mbd3*^{-/-} ES cells express these factors at uniformly high levels (Figure 1B). During development, wild-type ESCs silence these TFs in an Mbd3-dependent manner. Importantly, reduction of Klf4 or Klf5 levels by RNA interference results in partial rescue of the differentiation defect of *Mbd3*^{-/-} cells, suggesting that this phenotype results, in

part, from loss of the low-expressing ESC population and/or failure to fully silence these TFs under conditions promoting differentiation.

Interestingly, one of the factors promoting expression of self-renewal TFs, whose actions are opposed by NURD, is Stat3 (Reynolds *et al*, 2012), a key TF required for self-renewal in mouse ESCs (Young, 2011). A recent study from Crabtree and colleagues showed that the esBAF chromatin regulator facilitates Stat3-dependent transcriptional activation in ESCs (Ho *et al*, 2011). Consistent with these data, a separate study found that esBAF opposes NURD-mediated repression of several hundred target genes in ESCs (Figure 1A), resulting in moderate expression levels of these shared targets (Yildirim *et al*, 2011). Together, these findings suggest that the opposing functions of esBAF/Stat3 and NURD maintain variability in levels of key self-renewal TFs (and potentially numerous other regulatory factors) within the population of ESCs. This paradigm is reminiscent of the bivalent histone modifications found at many of the same genes in ESCs. The chromatin structure of most targets of polycomb complexes is marked by both the repressive histone modification, H3K27me3, and the activation-associated mark, H3K4me3. Thus, the imposition of opposing regulatory regimes to maintain moderate levels of gene expression appears to be a common regulatory strategy in ESCs. It will be interesting to learn how widespread this phenomenon is in other cell types and whether additional oppositional regulatory mechanisms are common.

Conflict of interest

The authors declare that they have no conflict of interest.

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